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PERSPECTIVE

Dangerous Liaisons: Connecting CRISPR/Cas9 to Clinical Science

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Recent advances in the field of gene editing have sparked a wave of enthusiasm in science and society. While potentially significant in the search to treat disease, the capacity to customize our genome brings with it a handful of ethical considerations. CRISPR/Cas9 is the latest—and allegedly the greatest—mechanism for gene editing to date. Through use of guiding RNA and nuclease activity, genes can be located and altered at a low cost. Though this technology has not yet reached an ethical experimental protocol useful for current medicine, the concept of clinical gene editing could alter the course of disease, if disease-causing genes could be eradicated or replaced. The model through which this profound technology is implemented is causing much controversy and discussion: that of the human embryo. In addition, issues related to editing the germline need a great deal of public deliberation before this technology is deployed.

Gene editing is the notion of using various nuclease-driven systems, such as transcription activator-like effector nucleases (TALENs), zinc-finger nucleases, or the most recent CRISPR-Cas9 system, to tailor a DNA sequence. These mechanisms work mostly through induced double-stranded breaks created at specific DNA binding sequences. These inducible breaks either allow the targeted gene to be taken out through nonhomologous end joining, or repaired through homology-directed repair (Ran et al., 2013). The TALEN and zinc finger systems have been used in many model organisms, and measures to increase their accuracy have progressed the field greatly in the last decade. Though well established in the genetic community, these methods are quickly becoming secondary to the Cas9 system as it rapidly accelerates its improvements.

The mechanism for the CRISPR/Cas9 system functions slightly differently. CRISPR specifically uses an RNA template combined with a nuclease to nick the sequence that is specified, and initiate repair mechanism to either delete or change that region. This procedure is more easily targeted to specific genes than the previous gene editing methods, and can be used to alter more than one gene at once. It is these assets to the CRISPR/Cas9 technology that could shed a new light on areas of medicine such as anticancer therapy, gene identification, and the ever-controversial topic of human germline editing (Gaj et al., 2013). While this ''search and replace'' system offers an incredible array of potential uses, it is currently not near the level of accuracy researchers and clinicians regard as ready for its clinical application. With this in mind, it is with caution that we address the bright future for clinical applications of the CRISPR/Cas9 system.

The current state of CRISPR/Cas9 research places us on the verge of successful human germline editing. Quite recently, a Chinese research group established a successful correction of a variant in the HBB gene known to cause β-thalassemia in humans using an RNA copy of the functional gene. While the study was moderately successful in its objective goals, it was not accomplished without error. Cautionary tales from many researchers working with the system address the ''off-target'' effects of the Cas9 nuclease, whose consequences are largely unknown. These off target hits are most likely caused by the high level of functionality of the Cas9 protein, posing a predicament in research goals that seek both powerful methods and efficacy. Not surprisingly, these off-target alterations were noted to occur in the embryos used in the study. Such off-target effects in mice have been noted to have minimal effects due to the lifespan of the organisms, but have been noted as the ''most critical criterion that needs thorough evaluation when performing genome editing'' when considering human embryonic alterations (Delerue et al., 2015). Further, the gene was repaired via the predicted mechanism for gene replacement via CRISPR, HDR, but problems arose regarding which genetic template the cell preferentially used to edit the nick: either the endogenous DNA or the CRISPR guide RNA. Lastly, in terms of viability, tripronuclear (TPN) embryos were used for this study, which are programmed to cease division at a certain stage. This deviation from the normal mechanisms of human development implies a limitation in how the long-term effects of the CRISPR/Cas9 implementation in embryos can be monitored (Fu et al., 2013). Combined with the discussed ''off-target'' edits, the long-term effects of CRISPR if done in embryonic vehicles are far from ascertained.

Perhaps the most obvious ethical consideration associated with the CRISPR/Cas9 system is the application of gene editing to medicine, specifically embryology. These edits can theoretically be tailored to cure disease-causing mutations, whether in somatic cells or in the germline, once the technology is sound and the resources are abundant. Nevertheless, with this new ease in editing comes ethical considerations of

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the most fundamental property of humanity: inheritance. Tweaking genes could give inheritance a lethal edge; passing down CRISPR-tailored genes could affect generations to come in ways we cannot predict with current models. What this recent study proves is exactly this: researchers specifically chose the TPN embryos because testing on legitimate human embryos is still ethically unfounded (Liang et al., 2015). Until safety is as guaranteed, clinical applications of this gene-editing system should not be regarded as a surefire science, but rather a distant reality that needs much more embellishment before coming to life.

With this step forward in science innovation, clinical applications and societal inferences need to take a deep breath. The more we learn about the CRISPR/Cas9 mechanism, the more the scientific community has interest in this powerful tool. While it isn't unreasonable to see this technology offered as a therapeutic in the future, it is necessary to understand the context for the tool's state of development. Use of this tool in humans must be preceded by public discourse and dialogue that may then result in an ethical system of regulation and experimentation. While we brace ourselves for the flood of possibilities CRISPR/Cas9 will undoubtedly release, it is critical to be cognizant at this moment in time that ethics must come first.

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